Pre-Lab Exercise:

- Read the “Measurements and Solutions” (pg. 67-69), “Making a Streak Plate” (pg. 74-76), and “Using Micropipettors” (pg. 77-79) sections of the Biology Student Handbook.
- Answer the following questions in your lab notebook:
  - How can we test the accuracy of a micropipette?
  - After plating your bacteria, why does each colony count as one cell?
  - What is the purpose of “bracketing” your dilutions?

Introduction:

As part of their daily routine, the microbiologist often has to determine the number of bacteria in a given sample, as well as having to compare the amount of bacterial growth under various conditions. Enumeration of microorganisms is especially important in medical microbiology, food and dairy microbiology, and environmental microbiology.

Concentrations of bacteria cells in samples are usually too great to be counted directly. Simple dilutions are practical when the dilution factor is about 100-fold or less. If the number of bacteria in a given sample is too great to be counted using a simple dilution, the sample is often serial diluted. Serial dilutions are a progressive series of dilutions. The final dilution is then plated out on an agar surface in such a manner that single isolated bacteria form visible isolated colonies that can be counted. The concentration of the original sample can then be back calculated based on the serial dilutions.

In our experiment, we want to determine the concentration of bacteria in our culture. We don’t know the actual concentration, but it is highly concentrated, on the order of $1 \times 10^8$ cells/mL. To get a countable number of colonies on our plates we want our 0.1mL sample to be at a concentration of approximately $1 \times 10^2$ cells/mL. This is a one million-fold dilution – clearly too big to do at once. We will solve this problem by doing a serial dilution.

Calculating Serial Dilutions

Serial dilutions are a way of dividing up a large dilution into a number of successive dilution steps with dilution factors of 100-fold or less. After you have determined the dilution factor needed to alter one concentration to another, the next step is to determine the practical means of making the actual dilution. There is a simple relationship between the dilution factor ($F_d$), the volume of the original concentrated sample to be diluted ($V_s$), and the total volume of the final dilute solution ($V_t$):

$$F_d = \frac{V_t}{V_s}$$

Remember that the final volume includes both the sample volume ($V_s$) and the volume of the dilutent to which it must be added ($V_d$). Therefore,

$$F_d = \frac{V_t}{V_s} \quad V_t = V_s + V_d \quad F_d = \frac{V_t + V_d}{V_s}$$
Notice that when F_d is determined, an infinite number of combinations V_s and V_t may be used to achieve that F_d. An F_d of 5 may be made by adding

\[
\frac{1 \text{ mL to } 4 \text{ mL}}{1} = (5 = 1+4)
\]

\[
\frac{0.1 \text{ mL to } 0.4 \text{ mL}}{0.1} = (5 = 0.1 + 0.4)
\]

Back to the example, to get the appropriate number of CFUs on a plate, you will need to dilute your sample using serial dilutions. Let’s assume the starting concentration is \(10^8 \text{ CFU/mL}\). If we dilute this sample by a dilution factor of \(10^5\) we get \(10^3 \text{ CFU/mL}\). By plating 0.1 mL of this dilution there are 100 CFU on the plate.

After the cells are spread on the plate, the plate is incubated and the cells grow into colonies. A colony contains millions of bacteria, but each colony arose from one cell so colonies are counted as one cell. The colonies are counted after 1-2 days of incubation. Once the colonies on the plate have been counted the approximate number of cells in the starting culture can be determined.

\[(\text{# of colonies/0.1 mL}) \times (\text{total dilution factor}) = \text{CFU/mL} \approx \text{Viable cells/mL}\]

The total dilution factor for a serial dilution is equal to the product of the individual dilution factors. For example if 97 colonies were counted in the example given above the number of CFUs in the starting culture are:

\[\text{CFU/mL} = (97/0.1 \text{ mL}) (10^3) = 9.7 \times 10^5 \text{ CFU/mL}\]

You do not know how many cells are actually in the original culture (after all, determining that number is the purpose of this exercise). You must, therefore, **bracket** your dilutions to be sure that you have at least some plates with a countable number of colonies, just as a photographer will make a range of exposures to be sure that at least one of the frames will have the proper amount of light to make a satisfactory photograph. For this exercise you should make plates at dilutions which are 10-fold less and 10-fold greater than the dilution which you expect to contain 100 CFU/0.1 mL.

If the starting number of cells is about \(10^8\), then a dilution factor of \(10^5\) will give the desired concentration of cells (\(10^3 \text{ cells/mL} = 10^2/0.1 \text{ mL}\)). If it is lower than \(10^8\), however, a dilution factor of \(10^5\) would give plates with only 10 colonies or less, which is not acceptable. In this case, a dilution factor of \(10^4\) would be more appropriate. Thus you must plan to make platings that will cover this situation, as well as if there are significantly more cells present. Plates made from dilution factors of \(10^5\), \(10^4\) and \(10^6\) should produce at least one set of "countable" plates.

Below is a diagram of a serial dilution scheme that should yield countable plates from a starting culture with \(10^8 \text{ cells/mL}\). Think through what is happening in each step until it makes sense to you!
Post Lab Assignment (Due by 5pm on 10/6/11):

1. Which dilution plate did you count?
2. What was the CFU count on this plate?
3. What was the concentration of bacterial cells in the original culture?